Conclusions: We have identified a series of potent, selective S1P1R antagonists, which may have the potential to be novel antivascular drugs for the treatment of cancer.

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Targeting MKP1 with novel chemical inhibitors sensitizes melanoma and colon cancer cells to chemotherapeutics in vitro and in vivo

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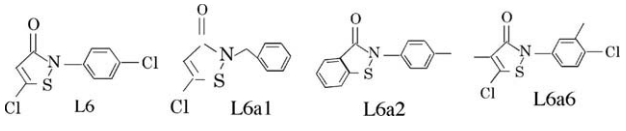
Phosphatase MKP1 is a putative cancer therapeutic target for inducing apoptosis and sensitizing cancer cells to chemotherapeutics, based on its over-expression in breast and lung cancer cells that is associated with an anti-apoptotic effect and chemo-resistance. However, chemical inhibitors of MKP1 with pre-clinical anti-tumor activity as a single agent or in combination with chemotherapeutics have not been reported. The significance of MKP1 in other malignancies (e.g., melanoma and colon cancer) remains undefined. From a library of drug-like small chemicals and chemical databases, we have identified lead compounds L6 and its more active analog L6a6 as novel MKP1 inhibitors with anti-cancer potential. Active against recombinant MKP1, the compounds selectively increased phosphorylation of MKP1 substrates in a dose-dependent manner (IC50 ~0.1 μg/ml) in melanoma and colon cancer cell lines in vitro and induced cell death (LD50 ~0.1 μg/ml) via apoptosis, in contrast to several structural analogs (e.g., L6a1) that had little effect on the substrates or cell growth. Consistent with its capacity to sensitize WM9 human melanoma cells to temozolomide (TMZ) or cisplatin in vitro, growth of WM9 xenograft tumors in mice was inhibited more efficiently (50%) by a tolerated combination of TMZ (80 mg/kg, ip) and L6a6 (10 mg/kg, oral) for two weeks (5 d/week) in comparison to TMZ alone (20%). L6a6 (3 mg/kg, oral, daily ×5 days/week for two weeks) also sensitized MC-26 colon cancer tumors in mice to 5-FU/LV, the standard regimen for colon cancer, inducing significantly better growth inhibition ($p < 0.01$) via combination (80%) than the chemotherapeutics (52%) or L6a6 alone (22%) in a tolerated manner. MKP1 expression in the cancer cell lines was verified by immunoblotting whereas high levels of MKP1 expression in advanced human melanoma tissues were detected by IHC. The compounds also sensitized WM9 melanoma cells to IFNa2b, a cytokine approved for melanoma treatment, and exhibited LD50 around 0.1 μg/ml toward human cell lines of breast cancer, lung cancer and prostate cancer in culture. In initial experiments with a promoter-insertion vector for up-regulating gene expression, L6a6-resistant WM9 clones have been isolated for mechanistic analysis. Taken together, these results provide direct evidence for the first time that support targeting MKP1 as a safe and efficacious cancer therapeutic strategy. Moreover, the pre-clinical anti-tumor activity of L6a6 as an orally active and well-tolerated lead compound suggests the potential of this class of chemicals for development into novel cancer therapeutics.



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GSK923925A, a novel and selective CENP-E inhibitor, induces pharmacodynamic effects and anti-tumor activity in human Colo205 xenografts

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Background: The mitotic kinesin centromere-associated protein E (CENP-E) integrates mitotic spindle mechanics with mitotic checkpoint signaling. CENP-E plays no known role outside of mitosis. CENP-E mRNA is over-expressed in a variety of human tumors relative to normal adjacent tissues suggesting it may play an important role in tumor cell proliferation. Inhibition of CENP-E in cultured human tumor cells leads to cell cycle arrest in mitosis with bipolar mitotic spindles and misaligned chromosomes and eventual cell death. GSK923295A is a novel and selective inhibitor of CENP-E ATPase activity that is currently in a Phase I clinical trial. The purpose of this study was to determine the pharmacokinetics (PK) and pharmacodynamics (PD) of GSK923295A in a human tumor xenograft model.

Methods: GSK923295A was administered intraperitoneally at 62.5, 125, or 250 mg/kg for two 3 day cycles separated by 4 days to nude mice with Colo205 tumor xenografts. Treated mice were divided into 2 cohorts: cohort 1 to monitor tumor growth and cohort 2 to determine PK in blood and tumor samples and PD (biomarkers) in tumor samples. PK was determined after the first dose of GSK923295A in each cycle.

Results: Tumor regression was observed at 125 and 250 mg/kg but no effect on tumor growth was observed at 62.5 mg/kg. Drug exposure was dose-dependent in both blood and tumors. An increase in drug exposure was observed in tumors, but not blood, between cycle 1 and 2. Examination of GSK923295A-treated tumors revealed a dose-dependent appearance of abnormal mitotic figures and a marked decrease in the presence of post-metaphase figures. Many of the abnormal mitotic figures had lagging chromosomes, a phenotype characteristic of CENP-E inhibition. GSK923925A also resulted in a dose-dependent increase in phosphohistone-H3 (pHH3) (a mitosis specific marker). The increase in pHH3 was transient and had largely disappeared by 48 h after dosing. PD effects were more pronounced after the second cycle of treatment with extensive tumor necrosis observed. The extent of pHH3 increase was related to the anti-tumor activity observed. At the lowest inactive dose, only a modest PD effect (increase in abnormal mitotic figures) was observed. At the active doses, more robust PD activity (both abnormal mitotic figures and increase in pHH3) was observed.

Conclusion: The observations made in vivo are consistent with previous cell based data and provide further insight into the potential of GSK923295A for the treatment of cancer.

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The ghrelin receptor agonist TZP-101 is a potent anti-tumor-cachexia agent in the human G361 melanoma mouse xenograft model

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TZP-101 is a small-molecule, ghrelin receptor agonist and prokinetic agent currently in Phase IIb clinical development for post-operative ileus and diabetic gastroparesis. Agonism of the ghrelin receptor has also been associated with increased food intake and the generation of a positive overall energy balance. The current study was intended to investigate the effect of TZP-101 as compared to ghrelin peptide on tumor cachexia in the G361 melanoma model grown as a subcutaneous xenograft in BALB/c nu/nu mice. Cachexia is considered the major reason for mortality, rapidly declining quality of life and limitation of therapy in advanced tumor patients. To this effect, 60 tumor-bearing mice were randomised 12 days after tumor cell inoculation into two sets of 5 groups containing 6 animals each. At initiation of treatment the average body weight loss of Set 1 (Groups 1−5) and Set 2 (Groups 6−10) animals was approximately 8.5% and 4.5%, respectively, of the initial average body weight. Treatment of Set 1 and 2 animals commenced on Days 12 and 16 after tumor inoculation, respectively. Groups 1 and 6 received vehicle s.c. bid alone, while Groups 5 and 10 were administered rat ghrelin peptide s.c. (1 mg/kg; bid, 6 h apart) as a positive control. TZP-101 was administered s.c. twice daily, 6 h apart, at doses of 3 (Groups 2 and 7), 10 (Groups 3 and 8) and 30 mg/kg (Groups 4 and 9) up to 33 (Groups 1−5) and 28 consecutive days (Groups 6−10). Mice were culled during the study according to predetermined criteria including >15% initial body weight loss and/or tumor volume in excess of 2000 mm3 and/or display of severe clinical signs.

As a result, TZP-101 treated animals of both Sets showed a dramatic increase in survival: while all vehicle treated control mice of Set 1 (Group 1) were dead on Day 5 after initiation of treatment, TZP-101 treated animals survived until Day 9, 28 and 30, at doses of 3, 10 and 30 mg/kg respectively. Similarly, the mean survival of Set 2 animals increased dose dependently from 17 days (vehicle treated controls) to 22, 26 and 27 days at TZP-101 doses of 3, 10 and 30 mg/kg, respectively. For comparison ghrelin treated mice survived for 33 (Set 1) and 22 days (Set 2). TZP-101 treatment was also associated with markedly increased food and water consumption, a clear tendency of increased body mass index, as well as dose dependently increased plasma concentrations of cholesterol, triglyceride and non-esterified fatty acids. In all cases treatment with TZP-101 caused a much greater response than ghrelin peptide. A concomitant 50% decrease in blood glucose levels may, in addition, support the notion of a change in metabolism.